

Effect of Zooid Spacing on Bryozoan Feeding Success: Is Competition or Facilitation More Important?

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Abstract. Most Recent bryozoan species are encrusting sheets, and many of these colonies have densely packed feeding zooids. In this study, I tested whether tight packing of feeding zooids affects food capture. Colonies of a bryozoan with an encrusting sheet form (*Membranipora membranacea*) were dissected to produce individuals whose feeding zooids were (1) closely packed, (2) more widely spaced, or (3) isolated. For each type, rates of particle ingestion were measured in still water and in a freestream velocity of 2.7 cm s^{-1} . Ingestion rate increased when zooids were closest together, probably because of reduced refiltration and increased feeding current strength farther from the lophophores. The mean incurrent velocity within 0.02 cm above the center of the lophophore was 0.28 cm s^{-1} regardless of zooid spacing; however, when the incurrent velocity was measured more than 0.1 cm from the lophophores, zooids that were close together or spaced one zooid's width apart had significantly faster incurrent velocities than single zooids. Flow visualization suggests that isolated zooids and those spaced far apart refilter more water than zooids that are close together. These results along with the observed trend of increased zooid integration over evolutionary time suggest that the benefits of increasing coordination outweigh the consequences of intrazooid competition.

Introduction

Bryozoan colonies can take many forms, but most Recent species are encrusting sheets (McKinney and Jackson, 1991). Whether the encrusting sheet has come to be the dominant bryozoan colony form because it is advantageous is unclear. Although the phylogeny of bryozoans is far from resolved, the encrusting sheet form is found across the

higher levels of the taxon, which suggests that convergence is likely. The growth pattern of an encrusting sheet is relatively simple and may also be relatively simple to evolve from multiple origins. While encrusting sheets are generally thought of as superior spatial competitors to runners (Buss, 1979b; Jackson, 1979; Bishop, 1989), the functional advantage of the encrusting sheet form has rarely been demonstrated experimentally.

The zooids in encrusting sheets are generally densely packed in a two-dimensional layer, and all of them stay in contact with the substratum (Fig. 1). This configuration has some potential benefits as well as costs. Since the zooids are on the substratum and do not project far from it, they experience the slower water velocities associated with the velocity gradient that exists close to the substratum. Therefore, the zooids are protected from the higher forces of fast flow, and they need little structural support. However, they can also experience a higher rate of sedimentation as well as a lower flux of food particles that result in a lower rate of food capture. The growth of encrusting sheets can theoretically be infinite, and in practice growth is generally limited only by the availability of substratum or food. For example, most encrusting colonies continue to grow as long as space and food are available, but some encrusting colonies, often called spots, show determinate growth and become reproductive at a very small size in habitats where space or food is limiting (Bishop, 1989; Okamura *et al.*, 2001). On the one hand, since the entire area of an encrusting colony is in contact with the substratum, the colony can take up a large amount of the available space and may be able to exclude competitors. On the other hand, encrusting sheets can be susceptible to overgrowth by some competitors. An advantage of having the feeding zooids close together in a sheet is the opportunity for coordinated feeding currents, but disadvantages of this configuration are the potential for more competition or for interference among the feeding currents of closely neighboring zooids. While any or all of these

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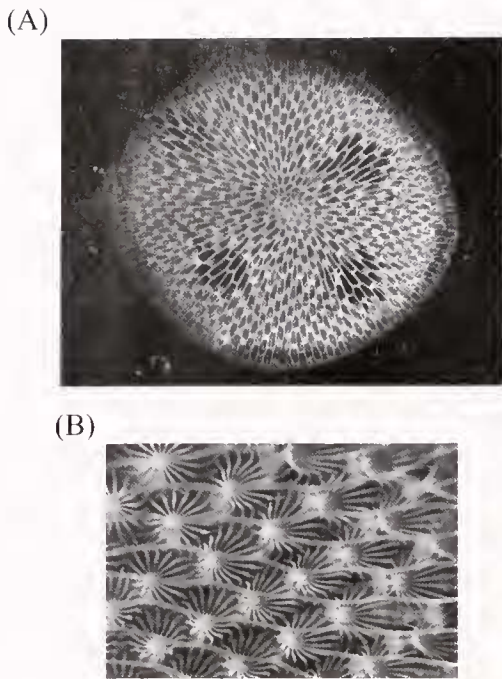


Figure 1. (A) Top view of a *Membranipora membranacea* colony. Note the darker spaces in the interior of the colony; these are the areas of excurrent flow called excurrent chimneys. (B) A closer top view of a group of feeding zooids with lophophores extended. Note that the lophophores are packed very tightly.

trade-offs could be important, the focus of this study is on the effect of zooid spacing on food acquisition.

Bryozoans use a ciliated crown of tentacles called a lophophore to create a feeding current that brings water laden with food down through the center of the crown toward the mouth and out through the base (Fig. 2). Food particles, which are generally phytoplankton, are transported to the mouth by a combination of mechanisms, including beat reversals of lateral cilia (Strathmann, 1973, 1982), tentacle flicks (Borg, 1926; Bullivant, 1968a; Strathmann, 1973; Larsen and Riisgård, 2002), other behaviors involving the tentacles (Winston, 1978), and bulk flow (Bullivant, 1968a; Best and Thorpe, 1983; McKinney *et al.*, 1986). Particle capture can be influenced by many factors such as temperature (Riisgård and Manríquez, 1997), particle concentration (Best and Thorpe, 1983, 1986b; Riisgård and Manríquez, 1997), presence of neighboring colonies (Okamura, 1984, 1985, 1988; Best and Thorpe, 1986a, b), particle size (Okamura, 1987, 1990), and colony size (Okamura, 1984, 1985).

Feeding zooids can show a wide variety of behaviors at the level of the individual (Winston, 1978, 1979; Shunatova and Ostrovsky, 2001) and the colony (Winston, 1978, 1979; Shunatova and Ostrovsky, 2002). At both levels, volumetric incurrent flow must equal volumetric excurrent flow according to the principle of continuity, where volumetric flow is equal to the velocity multiplied by the cross-sectional area

(Dick, 1987). Individual zooids, or those spaced very widely apart, do not experience interference in their incurrent or excurrent flows. In colonies where zooids are closer together, there may be substantial interference among the incurrent or excurrent flows. In encrusting sheets where zooids are packed tightly together, a canopy of lophophores creates a large incurrent space. The excurrent space is below the lophophore canopy, and water that enters this space will escape the colony at the periphery. As an encrusting sheet colony increases in size, the perimeter of the colony does not increase as fast as the total area, so the total cross-sectional area of the excurrent space decreases relative to the incurrent space. Having less excurrent area increases pressure in the interior of the colony, which could result in slower incurrent velocities and thus a decreased feeding rate. Some colonies stay small enough to have sufficient excurrent area on the perimeter relative to incurrent area; others create local excurrent spaces, called excurrent chimneys, in the interior of the colony (Fig. 1). The importance of having sufficient excurrent space in a colony is generally agreed upon, but how much is enough is not known, and whether there is substantial interference among feeding currents in the interior of a colony regardless of the amount of excurrent space remains unclear.

Mathematical models have been developed to determine whether or not neighboring feeding zooids interfere with or enhance each other's feeding currents. These two models complement each other in that one (Grünbaum, 1995) concentrates more on what happens to the flow below the lophophore canopy, while the other (Eckman and Okamura, 1998) focuses more on the flow above the colony. However, the conclusions the authors draw from their models differ. Grünbaum's (1995) model suggests that a large negative interaction in the excurrent flows in the area beneath the lophophore canopy increases the relative pressure in the

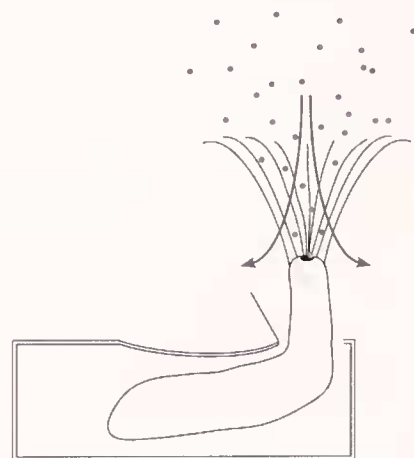


Figure 2. A schematic drawing of a single bryozoan zooid in side view. The small circles represent food particles (phytoplankton cells) that are being drawn down through the lophophore toward the mouth, which is at the base of the lophophore. Arrows indicate the direction of water flow.

interior of the colony. He concludes that this pressure below the lophophores should increase resistance to water flow and greatly reduce feeding currents. Thus, for this growth form to persist, other factors must make this colony form advantageous. In contrast, the model proposed by Eckman and Okamura (1998) suggests that as long as there is sufficient excurrent space, the combined ciliary currents produced by densely packed feeding zooids increase feeding rate. While both models agree that excurrent space is needed in the interior of a colony as it grows, controversy remains over the existence of interference or augmentation of feeding currents among zooids in encrusting sheets in nature. The purpose of this study was to test experimentally whether zooid spacing affects feeding rate. Since encrusting sheets are the dominant colony form and feeding success is crucial to acquiring the necessary energy for all other life processes, I hypothesize that closely packed zooids have greater feeding success than zooids that are spaced farther apart.

Materials and Methods

Bryozoan collection and maintenance

Membranipora membranacea (Linnaeus, 1767) usually grows as an encrusting sheet and is most often found on large macroalgae such as kelps. *Membranipora* was chosen for this study because it is tolerant of manipulations and its feeding zooids are densely packed (Fig. 1). Previous work indicates that *Membranipora* tends to have a higher ingestion rate than many other bryozoan species (Pratt, 2003), possibly due to the feeding advantage of its densely packed feeding zooids.

Colonies of *Membranipora* growing on the red alga *Mazzealla splendens* were collected from the floating docks at the Friday Harbor Laboratories in Friday Harbor, Washington. Large colonies were peeled off the algae, cut into pieces, and placed on glass slides (1 cm × 3 cm) in dishes of seawater. The colony pieces were allowed to grow onto the slides until they were firmly attached (usually 1–2 days). The slides were then placed in slide racks and hung off the floating docks so the bryozoans could feed in their natural habitat until needed for experiments. Colony pieces from the same original individual colony were recorded as being from the same clone and were later randomly assigned to different treatments.

Manipulations of zooid spacing

To create colonies with different spacing but the same total number of zooids, larger colony pieces were dissected down to eight zooids that were either spaced more widely ("spaced" treatment) or at their normal spacing ("close" treatment) (Fig. 3). To create the spaced treatment, all the surrounding zooids were dissected away (removing both the polypides and zooid walls with fine forceps) so that the

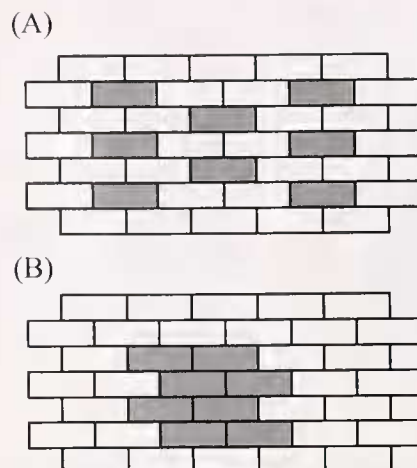


Figure 3. Schematic drawings of how dissections were performed to create the spaced (A) and close (B) zooid treatments (top view). The white boxes indicate where zooids were removed; the gray boxes indicate the live feeding zooids that were left untouched.

remaining eight zooids were each isolated and surrounded by a zooid's width of space on all sides. In addition to the spaced treatment with eight zooids in each treatment, I dissected some colonies down to a single zooid to represent a treatment in which there was no interference from neighboring zooids. Portions of colonies with zooids of a similar size were chosen for these dissections to eliminate potential effects of zooid size. To survive, bryozoan colonies must be able to tolerate damage from predators or abrasion. After neighboring zooids have been removed, the remaining zooids can bud and replace the removed zooids. Therefore, I have assumed that the dissections did not adversely affect the feeding ability of the remaining zooids once they were given a few days to recover from the dissections.

Feeding experiments

All feeding experiments were conducted in a recirculating flow tank (working section 70 cm × 10.2 cm × 18 cm, after Vogel and LaBarbera [1978]) filled to a depth of 13 cm with 0.45- μ m filtered seawater. Experiments were run at one of two freestream velocities, 0 and 2.7 cm s⁻¹, and the tank was kept in a cold room at 12 °C to maintain a constant water temperature similar to *Membranipora*'s natural habitat. Although some water motion usually remained in the tank, the ambient velocity was assumed to be near stagnant (0 cm s⁻¹) when the flow tank was turned off and allowed to settle for at least 10 min. The freestream velocity of 2.7 cm s⁻¹ (\pm 0.06 cm s⁻¹ SD) was estimated by taking the mean of velocities between 2.5 and 4.5 cm above the bottom. At the freestream velocity of 2.7 cm s⁻¹, there was a linear relationship between velocity (U , cm s⁻¹) and height above the bottom (z , cm) when $z < 1$ cm such that $U = (2.7) z$ ($R^2 = 0.95$) and shear velocity (U^* , where $U^* = (v \, dU/dz)^{1/2}$ (Vogel, 1994)) was 0.18 cm s⁻¹. Thus, the

ambient velocity in the horizontal direction at the top of an average lophophore (~ 0.11 cm) was 0.30 cm s^{-1} . Encrusting bryozoans such as *Membranipora* that live on large macroalgae most likely feed in the linear velocity gradient (or viscous sublayer) that exists close to the substratum (Lidgard, 1981; Grünbaum, 1995; Larsen *et al.*, 1998). Thus, the flow conditions used here are likely to be similar to what *Membranipora* colonies experience naturally.

The size and concentration of particles were chosen to be close to those quantified in the field and used in other studies (Bullivant, 1968b; Winston, 1979; Okamura, 1984, 1985; Pratt, 2003). The food particles used were blue-dyed polystyrene beads (mean diameter = $10.3 \mu\text{m}$, SD = $0.94 \mu\text{m}$, density = 1050 kg m^{-3}) at a concentration of $1000 \text{ beads ml}^{-1}$. Bryozoans have been shown to ingest large quantities of beads (Okamura, 1984, 1985), and observations of *Membranipora* zooids feeding on beads show that they do not reject the beads (pers. obs.). Concentrations were measured using a nanoplankton counting meter.

The glass slides were positioned flush with the bottom of the flow tank so only the bryozoans protruded into the flow. After feeding for 10 min, colonies were removed, fixed in formalin, rinsed in 70% EtOH, and cleared in 50% glycerol. The number of beads ingested by each zooid in the colony was counted using a compound microscope at $100\times$.

Flow visualization

Flow visualization was used to compare the incurrent velocities of the close, spaced, and single-zooid treatments when there was no ambient velocity. The water in the tank was seeded with particles that were about $20 \mu\text{m}$ in diameter and nearly neutral in buoyancy (cornstarch particles). To approximate a two-dimensional view, the flow field was illuminated with a 1-mm-thick laser light sheet (Lasiris SNF laser, 100 mW, 670 nm, focusable single line with 20° fan angle). Videos were recorded using a digital video camera (Sony DCR TRV900 with two +4 diopter close-up filters) with the optical axis perpendicular to the light sheet. The flow was analyzed by manually tracking particles from one video frame to the next. Only particles that entered the center of a lophophore were tracked. Once a particle was chosen, it was tracked from as far away from the lophophores as it was visible until it entered the lophophore. For the close and spaced treatments, particles were tracked for lophophores in the center of the colony.

Data analysis

Feeding rate with two spacing treatments and clone as a random factor. The first analysis of variance (ANOVA) tested the effect of zooid spacing and velocity on zooid ingestion rate for only the two treatments with eight zooids. There were not enough single zooids of the same clones as the close and spaced treatments to include the single treat-

ment in the analysis and still test the effect of clone, so the single treatment was excluded from this analysis. The dependent variable used in the ANOVA model was zooid ingestion rate, which is the number of beads ingested by a zooid divided by the length of the experiment (10 min). Since the dependent variable was measured on the zooid level, colony was used as a random blocking factor. I also used three different genetic individuals that were split into the different treatments, so clone was also included as a random blocking factor in the model. The data were analyzed with a mixed ANOVA model (SAS 8.02, PROC MIXED) where spacing (close and spaced) and velocity (0 and 2.7 cm s^{-1}) were classified as fixed effects, and clone and colony were classified as random effects.

Feeding rate with all three spacing treatments. The second ANOVA was similar to the first except that clone was excluded as a random factor and the single zooid treatment was added as a level in the fixed spacing factor. *A priori* and *a posteriori* tests were also included in the analysis. Orthogonal polynomial tests can be used to run what is essentially a regression on treatment means to test for trends in the dependent variable as a function of the treatment effects (Quinn and Keough, 2002). *A priori* orthogonal polynomial tests were performed to assess whether zooid ingestion changed as a linear or quadratic function of zooid spacing. *A posteriori* pairwise comparisons were performed using the Tukey-Kramer adjustment.

Flow visualization. Incurrent velocity as a function of distance from the lophophores was measured for four particles for each of two colonies for each spacing treatment at 0 cm s^{-1} . Mixed-model analysis of covariance (ANCOVA) tests (SAS 8.02, PROC MIXED) were used to compare how the different spacing treatments (close, spaced, and single) affected the incurrent velocity with distance from the lophophore as the covariate. First, the slopes were compared using the following model:

$$Y_{ijkm} = \mu + \alpha_i + \beta_i X_{ijkm} + c_{j(i)} + d_{k(j(i))} + \varepsilon_{ijkm} \quad (\text{Equation 1})$$

where Y_{ijkm} = the value of the response variable (incurrent velocity in cm s^{-1}), μ = the overall mean incurrent water velocity, i = the levels of the spacing factor (1 = close, 2 = single, 3 = spaced), j = the number of colonies (two colonies per spacing treatment), k = the number of particles tracked (four particles per colony per spacing treatment), m = the observations of particle velocity at a particular distance from the lophophores, α_i = the intercept (fixed effect) of the i^{th} spacing treatment, β_i = the slope for the i^{th} spacing treatment, X_{ijkm} = the distance from the lophophore (cm) of the m^{th} observation of the k^{th} particle in the j^{th} colony of the i^{th} spacing treatment, $c_{j(i)}$ = the random effect of the j^{th} colony in the i^{th} spacing treatment, $d_{k(j(i))}$ = the

random effect of the k^{th} particle of the j^{th} colony in the i^{th} spacing treatment, and ε_{ijkm} = the unexplained random error. If the slopes had not been found to be significantly different (*i.e.*, if $P > 0.05$), then the slopes among spacing treatments would have been set as equal to each other, and the intercepts would have been compared using the following:

$$Y_{ijkm} = \mu + \alpha_i + \beta X_{ijkm} + c_{j(i)} + d_{k(j(i))} + \varepsilon_{ijkm}, \quad (\text{Equation 2})$$

where β is the common slope for all levels of the spacing factor. However, since the slopes differed, the data were split into three subset ranges of the covariate: (1) $0 < D \leq 0.05$, (2) $0.05 < D \leq 0.1$, and (3) $0.1 < D \leq 0.15$ where D is the distance (in cm) from the lophophore. ANCOVA tests using Equation 2 were done separately on each of the three subset ranges, and the Tukey-Kramer adjustment was used for *a posteriori* pairwise contrasts. Incurrent velocities were log-transformed in all analyses.

Results

Feeding experiments

Feeding rate with two spacing treatments and clone as a random factor. The zooids in the close treatment had significantly higher mean zooid ingestion rates than the zooids in the spaced treatment, but velocity and the interaction between velocity and zooid spacing did not have a significant effect on ingestion rate (Fig. 4). A significant amount of variation in zooid ingestion rate was contributed by the colony and clone random factors ($P < 0.0001$).

Feeding rate with all three spacing treatments. Spacing and velocity had significant effects on zooid ingestion rate, but

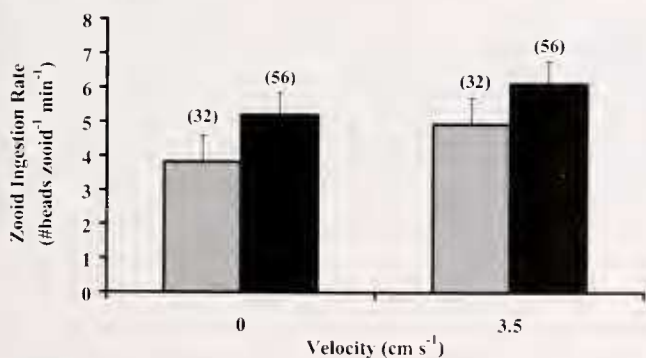


Figure 4. Zooid ingestion rate in two free-stream velocities for two zooid spacing treatments. Spaced zooids: gray bars; close zooids: black bars. Ingestion rate was greatest for closely spaced zooids ($P = 0.0361$). Velocity ($P = 0.1157$) and the interaction between velocity and spacing ($P = 0.8697$) did not significantly affect ingestion rate. Bars represent the least squares means \pm one standard error of the least squares mean, and the number of zooids in each treatment is shown in parentheses.

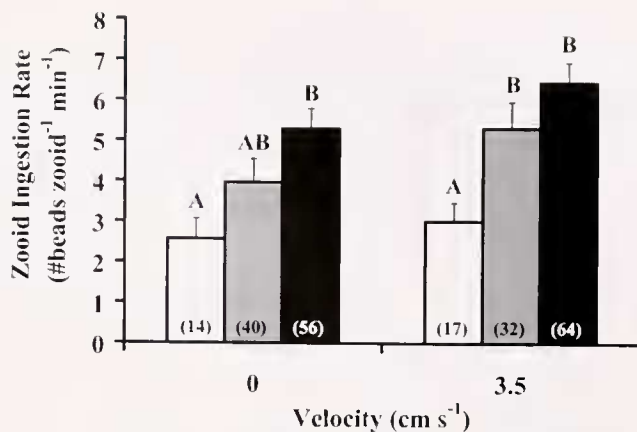


Figure 5. Zooid ingestion rate in two free-stream velocities for three zooid spacing treatments. Single zooids: open bars; spaced zooids: gray bars; close zooids: black bars. Overall, spacing ($P < 0.0001$) and velocity ($P = 0.0293$) significantly affected ingestion rate, whereas the interaction between spacing and velocity did not have a significant effect ($P = 0.6562$). Ingestion rate increased linearly ($P < 0.0001$) but not quadratically ($P = 0.5405$) as zooids were spaced closer together. Bars with the same letter were not significantly different from each other in pairwise comparisons (see Table 1 for statistics). Bars represent the least squares means \pm one standard error of the least squares mean, and the number of zooids in each treatment is shown in parentheses.

the interaction between spacing and velocity did not (Fig. 5). As in the above analysis, the random colony factor significantly increased the variation in ingestion rate ($P < 0.0001$). Mean zooid ingestion rate was significantly higher at 2.7 cm s^{-1} than at 0 cm s^{-1} when spacing treatments were pooled, but ingestion rate was not significantly different between velocities within any of the spacing treatments (Table 1). There was a significant linear trend in spacing: zooid ingestion rate increased as zooids were spaced closer together (Fig. 5). Both the close and spaced treatments had significantly greater mean zooid ingestion rates than single zooids when velocity treatments were pooled (Table 1). However, when the data were analyzed for each velocity separately, the differences among spacing treatments were not quite as strong as when velocity treatments were pooled (Table 1). Both the single and spaced zooids had all the zooids surrounding them removed, yet the spaced zooids had significantly higher mean ingestion rate than the single zooids, which suggests that the dissection process itself did not adversely affect feeding performance.

Flow visualization

ANCOVA tests revealed that the two random factors, colony and particle, each significantly increased the variation in incurrent velocity ($P < 0.0001$). Incurrent velocity scaled significantly differently with distance from the lophophores among treatments (Fig. 6), so the data were divided into three subset ranges of distance from the lophophores: (1) $0-0.05 \text{ cm}$, (2) $0.05-0.10 \text{ cm}$, and (3) $0.10-$

Table 1

A posteriori pairwise comparisons testing the effects of zooid spacing and water velocity on zooid ingestion rate

	Spacing pooled	Within-spacing treatments		
		Close	Spaced	Single
0 vs. 2.7 cm s ⁻¹	0.0293	0.5579	0.6340	0.9863
	Velocity pooled	Within-velocity treatments		
		0 cm s ⁻¹	2.7 cm s ⁻¹	
Close vs. Spaced	0.0782	0.5008	0.7234	
Close vs. Single	< 0.0001	0.0045	< 0.0001	
Spaced vs. Single	0.0048	0.4614	0.0510	

Three zooid spacing treatments (close, spaced, single) and two velocities (0, 2.7 cm s⁻¹) were used in this analysis. Zooid ingestion rate is the number of beads eaten per zooid per minute. All values in the table represent the *P* value for the given pairwise comparison.

0.15 cm. Separate ANCOVA tests on each subset revealed that spacing had an increasingly significant effect on incurrent velocity when the particles were further away from the lophophores (Table 2). Pairwise *a posteriori* tests revealed that the close and spaced treatments had significantly faster incurrent velocities than the single zooid treatment when the

particles were greater than 0.1 cm from the lophophores, but this difference disappeared as the particles got closer to the lophophores (Table 2). Conversely, the incurrent velocities of the close and spaced treatments never differed significantly (Table 2). Since the incurrents very close to the lophophores (within 0.05 cm) were never significantly different among any of the spacing treatments (Table 2), the assumption that the dissections did not affect zooid feeding is supported. The mean incurrent velocity within 0.02 cm of the lophophore for all three treatments was 0.28 cm s⁻¹ (\pm 0.08 cm s⁻¹ SD).

Discussion

Tightly spaced zooids have a feeding advantage

The results of this study demonstrate that ingestion rate increases when zooids are closer together. This difference was especially strong when the ingestion rates of isolated zooids were compared with the ingestion rates of the closely spaced zooids in natural *Membranipora membranacea* colonies. Thus, these results support the conclusions of Eckman and Okamura (1998) rather than those of Grünbaum (1995).

At least in small colonies, what happens above the colony (refiltration and the incurrent velocity far from the lophophores) may thus be more important than what happens

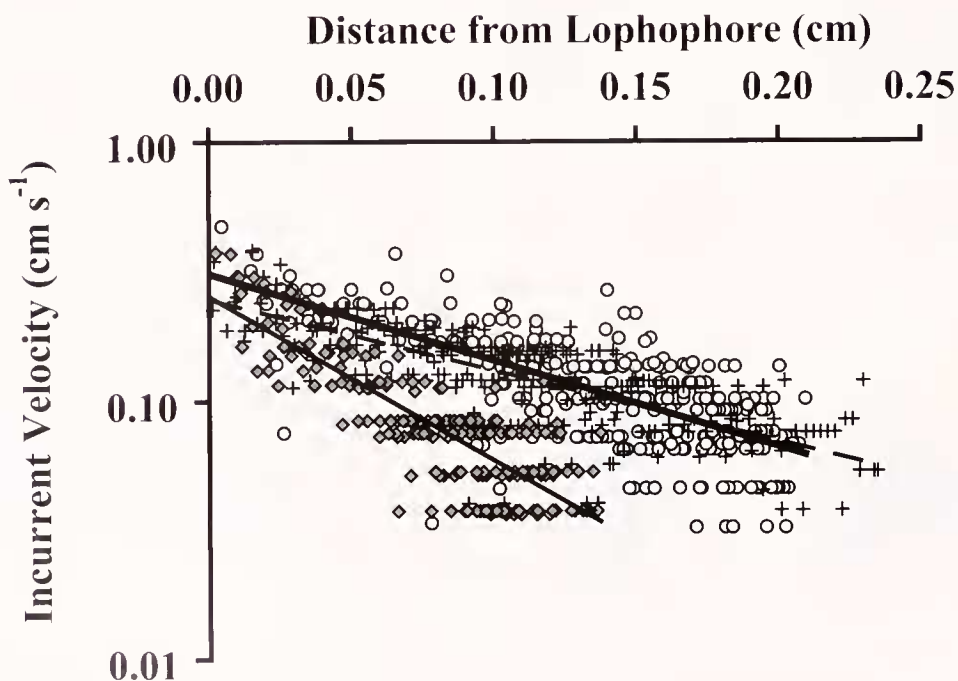


Figure 6. Velocity of particles traveling in the incurrent flow of bryozoan zooids, measured as a function of distance from the lophophores for three zooid spacing treatments. Close zooids: plus signs, thick dashed line; spaced zooids: open circles, thick solid line; and single zooids: gray diamonds, thin solid line. Lines represent standard least squares regressions for each treatment (close: $\ln y = -6.33x - 1.40$; spaced: $\ln y = -7.72x - 1.17$; single: $\ln y = -14.63x - 1.37$). Analysis of covariance revealed significant differences among the slopes of these lines ($P < 0.0001$).

Table 2

A posteriori analysis of covariance comparing the effects of zooid spacing on log-transformed incurrent velocity as a function of distance from the lophophore

		0 < D ≤ 0.05	0.05 < D ≤ 0.1	0.1 < D ≤ 0.15
Type 3 Tests of Fixed Effects	Spacing	0.5427	0.0516	0.0252
	Distance	< 0.0001	< 0.0001	< 0.0001
Pairwise Contrasts	Close vs. Spaced	0.8340	0.7200	0.6484
	Close vs. Single	0.6790	0.0646	0.0435
	Spaced vs. Single	0.5429	0.0673	0.0254

Three zooid spacing treatments (close, spaced, single) were used in this analysis. All values represent *P* values. *D*, distance from the lophophore (cm).

below the lophophore canopy within the colony. This is not to say that the pressure that can build up under the lophophore canopy is not important; indeed, both mathematical models agree that, as a colony grows, excurrent chimneys are needed to keep some balance between incurrent and excurrent flow. However, as long as there is enough excurrent space to relieve the pressure built up under the lophophore canopy as the colony grows, then external conditions and refiltration seem to be more important.

Consequently, the feeding advantage of tightly packed zooids most likely results from a reduction in refiltration and an increase in the strength of the feeding current farther from the lophophores (Eckman and Okamura, 1998). If excurrent water is reintroduced above the lophophore and mixes with the incurrent water, the concentration of particles in the incurrent water will be reduced. Avoiding refiltration of excurrent water should increase the particle flux through the lophophores and increase the particle ingestion rate. In addition, increased incurrent velocity farther from the lophophores should result in increased particle capture because the feeding current reaches farther into the water column to regions of higher particle flux due to faster ambient water velocities and increased turbulent mixing.

This study provides evidence of both reduced refiltration and increased feeding current strength farther from the lophophores. Because incurrent velocities were never significantly different with height between the close and spaced zooids, the most likely explanation for the greater ingestion rate measured in the close zooids is that less refiltration occurs when zooids are closer together. Overall, zooids spaced farther apart may be "leakier," so that more particles escape between them. Flow visualization revealed that particles approaching the outer edges of the lophophore often escaped between or just above the tips of the tentacles, but when zooids were close together, neighboring zooids often caught the escaping particle. When zooids were farther apart, many particles were observed to slip between zooids uncaught (Fig 7). Both the spaced and close zooids had significantly higher zooid ingestion rates than the single zooids; and while this may be partly a result of greater refiltration by isolated zooids, it is also likely to be due to

slower incurrent velocities for the single-zooid treatment when a particle was more than 0.1 cm from the lophophores (Figs. 6, 7). Flow visualization revealed that at the freestream flow of 2.7 cm s^{-1} , the spaced and close treatments both could capture particles up to 0.07 cm above the lophophores (traveling downstream at $\sim 0.49 \text{ cm s}^{-1}$), while the isolated zooids could only capture particles up to 0.04 cm above them (traveling downstream at $\sim 0.41 \text{ cm s}^{-1}$) (Fig. 7). Thus, isolated zooids have effectively weaker incurrents than those of the zooids in the spaced and close treatments.

These results support the assumption that closely spaced zooids create faster, more vertically directed feeding currents (Figs. 6, 7). Models of bryozoan feeding currents have assumed that the shape of the incurrent water flow over an isolated zooid is an inverted cone, whereas the shape over zooids that are tightly packed is cylindrical (Grünbaum, 1995; Eckman and Okamura, 1998). To conform with the principle of continuity (Vogel, 1994), the velocity at a given distance from the lophophore should be lower in a conical incurrent than in a cylindrical one. Similarly, measurements of the feeding current velocities in *Electra pilosa* suggest that the combined feeding currents of two lophophores are faster and more vertically directed than the currents of an isolated lophophore (Larsen and Riisgård, 2002).

My data on incurrent velocities agree well with other published measurements of *Membranipora* colonies. The average incurrent velocity less than 0.02 cm from the lophophore for all treatments in this study was 0.28 cm s^{-1} . Values of incurrent velocities cited in the literature for *Membranipora* include a mean of 0.25 cm s^{-1} (Lidgard, 1981) and a maximum of 0.24 cm s^{-1} (Larsen and Riisgård, 2001) for zooids in a colony, as well as a mean and standard deviation of $0.258 \pm 0.08 \text{ cm s}^{-1}$ for isolated zooids (Riisgård and Goldson, 1997).

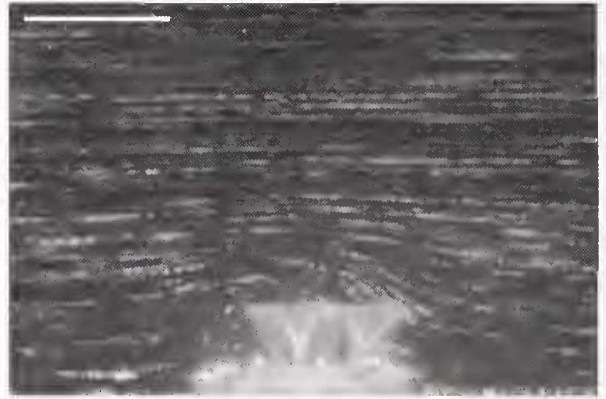
Importance of spacing and flow velocities to suspension feeders

Although this is the first time that the effect of zooid spacing on feeding success has been tested in bryozoans,

(A) (i)



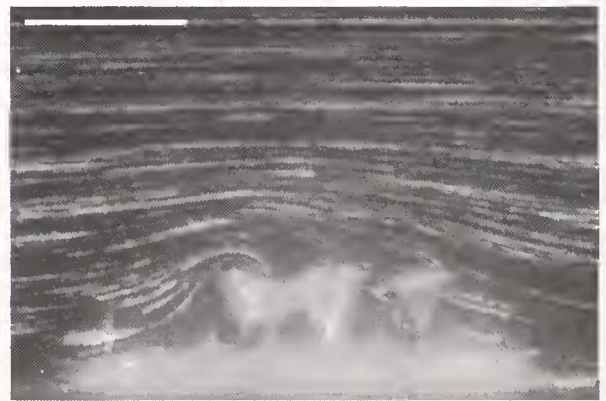
(A) (ii)



(B) (i)



(B) (ii)



(C) (i)



(C) (ii)



Figure 7. Example of flow around colonies at (i) 0 cm s^{-1} and (ii) 2.7 cm s^{-1} free-stream velocity. Colonies were dissected to create three treatments: (A) 8 zooids close together, (B) 8 zooids further apart, and (C) isolated zooids. These pictures were created by overlapping 180 frames of video ($\sim 6 \text{ s}$) using a macro in Scion Image ver. 3b (macro developed by M. von Dassow, University of California Berkeley). Images are taken from the side with freestream flow (when 2.7 cm s^{-1}) coming from the left to right. Scale bars are 0.2 cm.

studies on other suspension feeders have investigated the effects of individual or zooid or polyp spacing on feeding success. For example, phoronids are not colonial, but these lophophorate suspension feeders can live in very dense aggregations (up to $20,000 \text{ m}^{-2}$) (Johnson, 1997). On the

basis of studies of growth and of flow around neighboring phoronids, Johnson (1990, 1997) predicted increased feeding success when there is an upstream neighbor; however, this advantage disappeared when the upstream neighbor was within one lophophore's diameter ($\sim 1 \text{ cm}$).

The presence of neighbors is also important for nonciliary suspension feeders. For example, upstream neighbors were found to decrease prey capture in the sea anemone *Metridium senile* (Anthony, 1997). One species of acorn barnacle, *Semibalanus balanoides*, had higher particle capture rates in the middle of a dense aggregation than in solitary individuals or those on the edge of a dense aggregation (Bertness *et al.*, 1998); another acorn barnacle, *Balanus amphitrite*, had higher particle capture rate upstream and at the peak of hill-shaped clusters than did barnacles located downstream (Pullen and Labarbera, 1991). Having different species as neighbors also may influence feeding success. Feeding performance was higher in mixed assemblages of three species of hydroptychid caddisfly larvae than in species monocultures (Cardinale *et al.*, 2002).

The spacing of neighbors or polyps can have different effects depending on the ambient flow velocity. The influence of neighbor colonies on feeding rate has been investigated in both arborescent and encrusting colonies of bryozoans in different flow velocities. The presence of a large upstream neighbor decreased the feeding rate of small conspecific colonies of an arborescent bryozoan (*Bugula stolonifera*) in both a relatively slow ($1\text{--}2\text{ cm s}^{-1}$) and a fast ($10\text{--}12\text{ cm s}^{-1}$) ambient velocity (Okamura, 1984). Conversely, the presence of a neighbor colony 1–5 mm upstream increased the feeding rate of conspecific colonies of an encrusting bryozoan (*Conopeum reticulum*) in a relatively slow velocity ($1\text{--}2\text{ cm s}^{-1}$) (Okamura, 1985). Encrusting colonies of *Electra pilosa* have reduced feeding rates when surrounded by *Alcyonidium hirsutum* colonies at a relatively slow flow ($1\text{--}2\text{ cm s}^{-1}$), but they have increased rates when surrounded by *A. hirsutum* or *Flustrellidra hispida* colonies at a relatively fast flow ($10\text{--}12\text{ cm s}^{-1}$) (Okamura, 1988). Similarly, dense aggregations of branches of the scleractinian coral *Madracis mirabilis* have high food capture at higher flow rates, but more spaced aggregations of branches have their highest food capture rate at intermediate flow velocities (Sebens *et al.*, 1997).

Previous experiments on medium-sized ($0.51 \pm 0.19\text{ cm}^2$) *Membranipora membranacea* colonies suggest that the zooid ingestion rate increases until the average free-stream velocity reaches about 2.7 cm s^{-1} , and then decreases (Pratt, 2003). The results of this study also suggest that the ingestion rate increases when velocity increases from 0 to 2.7 cm s^{-1} . If additional velocities between 0 and 2.7 cm s^{-1} were tested, I would expect a significant linear trend in zooid ingestion rate as a function of velocity. Although ambient velocity did not alter the significance of spacing to relative ingestion rate in this study, it may be that differences would occur at a wider range of flow. Alternatively, colonies can respond to flow with phenotypic plasticity, in which zooids become smaller in faster flow (Okamura and Partridge, 1999); however, the colony must be exposed to that velocity for a long time.

Implications and significance of zooid spacing

Although the results of this study suggest that ingestion rate increases with decreased distance between zooids, the relevance of this phenomenon to the fitness of bryozoan colonies remains to be demonstrated. There is some evidence that *Membranipora* has a higher feeding rate and thus a higher growth rate than other native species in the Pacific Northwest (Pratt, 2003). This relatively high feeding rate may be a consequence of the efficient colonywide filter created by closely packed zooids. Perhaps the high feeding rate and growth rate of this species have contributed to its fast and effective invasion of the Gulf of Maine (Berman *et al.*, 1992; Lambert *et al.*, 1992; Scheibling *et al.*, 1999).

While the results of this study show that having zooids close together can enhance feeding performance in very small colonies, whether or not this advantage is important in larger colonies is not known. Larger colonies may have higher fitness since fecundity (Hayward, 1973; Hayward and Ryland, 1975; Winston and Jackson, 1984; Jackson and Wertheimer, 1985), growth rate (Lutaud, 1983; Winston and Jackson, 1984; Hughes and Hughes, 1986), and survivorship (Sutherland and Karlson, 1977; Buss, 1981; Russ, 1982; Winston and Jackson, 1984) increase with colony size. Still, increasing colony size must cost something, and some colonies show an upper size limit (spots: Bishop [1989]; Okamura *et al.*, [2001]; plates: Nielsen [1981]; erect branching colonies: Cheetham and Hayek [1983]; Cheetham [1986]). As an encrusting sheet colony grows past a certain size, internal excurrent space is necessary to balance the incurrent and excurrent flows. In fact, chimneys may form in the internal areas of the colony where the per-zooid feeding rate is lowered as a result of the building backpressure under the lophophore canopy as the colony grows (Larsen and Riisgård, 2001). The consequences of these excurrent spaces for feeding performance are not clear. Refiltration of water can decrease particle flux and lower particle capture rates in bryozoans (Grünbaum, 1995; Eckman and Okamura, 1998; Larsen *et al.*, 1998; Larsen and Riisgård, 2002; this study). Concentrating the excurrent flow into a fast jet of water may help prevent refiltration (Lidgard, 1981; M. Pratt, unpubl. data); however, as ambient flow increases, excurrent jets from chimneys may be directed back toward the colony surface and no longer prevent refiltration (M. Pratt, unpubl. data). Ambient flow conditions and how the feeding currents interact with the ambient flow are clearly important in determining bryozoan feeding performance (Lidgard, 1981; Eckman and Okamura, 1998; Larsen *et al.*, 1998; Okamura *et al.*, 2001; Larsen and Riisgård, 2002).

Once a sessile animal such as a bryozoan settles, it cannot relocate to a new habitat if conditions become suboptimal. However, a colony may adapt its shape or size to optimize conditions for feeding. Okamura and Partridge's (1999) study revealed not only that colonies can have smaller

zooids in faster flows to maintain a favorable velocity at the level of the colony, but that these colonies have the same growth rate as colonies with larger zooids in slower flows. Further research exploring the effects of factors such as colony shape and size as well as a wider range of flow conditions on feeding would enhance our understanding of the relationship between feeding performance and surrounding flow conditions.

The fossil record of bryozoans shows an increase in zooid integration over evolutionary time (McKinney and Jackson, 1991), and this study provides evidence for one functional explanation for selection in favor of integrated feeding currents. Traditionally, spatial limitation (Buss, 1979a; Jackson, 1979), dislodgement risk (Cheetham and Thomsen, 1981; Cheetham, 1986), and predation (McKinney and Jackson, 1991) have been identified as major selective factors driving the evolution of colony form in bryozoans. It is becoming increasingly clear that food acquisition also has played a significant role in the evolution of colony form (Eckman and Okamura, 1998; Okamura *et al.*, 2001; this study).

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